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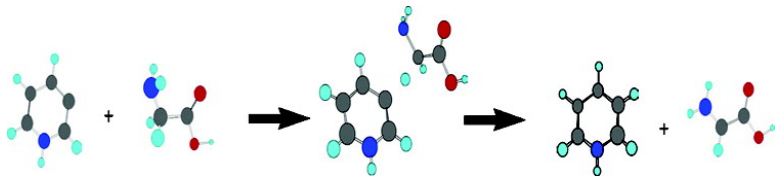

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Reactions of Charged Phenyl Radicals with Aliphatic Amino Acids in the Gas Phase

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Abstract: Gas-phase reactivity of five differently substituted positively charged phenyl radicals was examined toward six amino acids by using Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR). The reactivity of the radicals studied was determined by the electrophilicity of the radical, which can be characterized by the radical's electron affinity (EA). The larger the electron affinity of the radical, the higher the overall reaction rate. In addition to the expected H-atom abstraction, several unprecedented reaction pathways were observed, including NH_2 abstraction, SH abstraction, and SCH_3 abstraction. These reaction pathways dominate for the most electrophilic radicals, and they may not follow radical but rather nucleophilic addition−elimination mechanisms. Hydrogen abstraction from glycine was also investigated theoretically. The results indicate that hydrogen abstraction from αC of glycine is both kinetically and thermodynamically favored over the NH_2 group. The ordering of transition state energies for hydrogen abstraction from the αC and NH_2 groups was found to reflect the radicals' EA ordering.

Introduction

Reactions of oxygen-containing radicals (such as hydroxyl, peroxyl, and alkoxyl radicals) with proteins can cause cross-linking, fragmentation, side-chain oxidation, denaturation, and loss of enzymatic activity of the protein.[1−5] Numerous studies have been carried out to explore the reactivity of small radicals, such as HO, toward proteins, peptides, and amino acids in solution.[1−13] Study of the radical reactions of amino acids and small peptides can offer useful information on the radical reactions of proteins because amino acids and peptides are the building blocks of proteins. Various sophisticated techniques (such as electron spin resonance spectroscopy[13]) have been utilized in these studies. Solution studies of reactions of the HO radical with amino acids have shown that hydrogen abstraction from αC or an alkyl group is the predominant reaction pathway for amino acids that do not have aromatic or sulfur-containing side chains.[1,13] A significant portion of the abstracted hydrogen atoms also comes from the amino group,[11] although the αC−H bond is significantly weaker than the N−H bond.[14] For amino acids containing aromatic rings (phenylalanine, tyrosine, tryptophan, and histidine), addition to the aromatic ring appears to be the major reaction pathway.[1] In methionine and cysteine, the HO radical adds to the sulfur atoms.[1] A particularly fast hydrogen abstraction from the SH group of cysteine has also been observed.[1]

Literature evidence suggests that in addition to small oxygen-containing radicals, also some carbon-centered organic radicals (such as the phenyl radical and related biradicals) can cause protein damage.[15a,15b] However, in sharp contrast to the extensive studies carried out on small oxygen-containing radicals to better understand their mechanisms of protein degradation, the reactions of organic radicals with proteins are nearly entirely unexplored. To the best of our knowledge, only one mechanistic study has appeared in the literature on reactions of phenyl radicals with proteins, peptides, or amino acids—and in this study, only glycine was examined.[15b] However, this study demonstrated unambiguously that *para*-benzoic acid radicals (4-dehydrobenzoic acid) can abstract deuterium from the α-position of α,α-dideuterioglycine, and are therefore also likely to be able to damage proteins. Phenyl radicals and biradicals are generated in biological systems as intermediates of some potential drug candidates that target DNA.[16a,16b] Obviously, the radical intermediates' side-reactions with proteins, which may cause cytotoxicity and other unwanted side effects, are of great interest.

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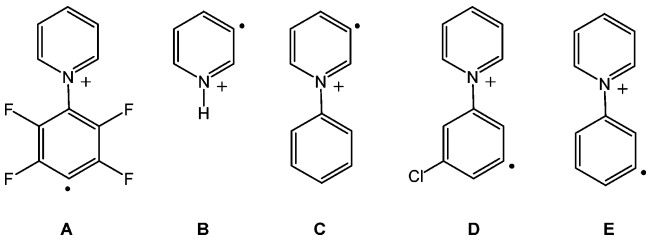

Figure 1. Positively charged phenyl radicals used in this study.

Therefore, further studies into the ability of phenyl radicals to damage amino acids, peptides, and eventually proteins, are clearly warranted.

Exploration of radical reactions with amino acids and peptides in the gas phase can provide complementary information to condensed phase data. During the last several years, our laboratory has advanced a "distonic ion approach" to examine substituted phenyl radicals' reactions in a Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR).[17] This approach is based on the attachment of a chemically inert charged group, usually pyridinium, to the radical of interest for mass spectrometric manipulation and detection. The examination of radical reactions in a mass spectrometer has several advantages. Isolation of the charged radicals is fast and easy because the low-pressure conditions of a mass spectrometer guarantee that only very few collisions occur between these highly reactive species and other molecules. Second-order reaction rate constants are readily measured for radical reactions. Solvation effects do not perturb the observed radical reactivity. We report here an experimental and computational examination of the reactions of five charged phenyl radicals with six aliphatic amino acids.

Experimental Procedures

Most of the experimental procedures used in this work have been described previously.[17] The experiments were performed using a Finnigan model FTMS 2001 dual-cell Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR). The instrument consists of a differentially pumped dual-cell reaction chamber aligned collinearly within a magnetic field generated by a superconducting 3.0 T magnet. The vacuum in the instrument is maintained by the use of two Edwards diffusion pumps (800 L s^{-1}), placed in both sides of the instrument, each backed with an Alcatel mechanical pump. The nominal baseline pressure of each cell is $\leq 1 \times 10^{-9}$ Torr, as measured by ionization gauges located on each side of the dual cell. Both sides of the instrument are equipped with inlets for liquid samples and with a probe for introduction of solid and/or nonvolatile chemicals.

All the amino acids were introduced into the instrument by using a thermal solids probe. Most amino acids have high melting points, and some decompose at temperatures below their melting point. However, the amino acids studied here could be introduced into the instrument without thermal decomposition. The formation of an abundant protonated molecule upon the reaction with protonated acetone was used to verify that the thermally introduced amino acids remain intact.

The generation of the phenyl radicals has been described previously.[17] The procedure for the formation of the *N*-phenyl-3-dehydropyridinium ion (radical C, Figure 1) is briefly described here. 3-Iodopyridine and bromobenzene were introduced into the same cell of the instrument via a Varian leak value and a batch inlet, respectively. A 20 eV electron beam was used to ionize both compounds, yielding abundant molecular ions as well as various fragment ions. The bromobenzene radical cation generated during electron ionization was allowed to react with 3-iodopyridine to form the *N*-phenyl-3-iodopyridinium cation by *ipso*-substitution of a Br atom with 3-iodopyridine. The *N*-phenyl-3-iodopyridinium cation was then transferred into the other cell (for the future reaction with neutral amino acids) by grounding the conductance limit plate (a common wall between the two cells) and was isolated by ejecting all unwanted ions from the cell. Sustained off-resonance irradiated collision-activated dissociation (SORI-CAD)[18] was employed to homolytically cleave off the iodine atom to generate the radical C. This was performed by gently exciting the ions for 0.3−1 s by applying an rf pulse that was slightly off-resonance with the ion cyclotron frequency (~1 kHz) under a relatively high pressure of a collision gas (Ar at a nominal pressure of 1×10^{-5} Torr). The radicals A, B, D, and E (Figure 1) were formed using a similar procedure except that different ion precursors were used (pyridine and 1,4-diiodotetrafluorobenzene for A, 3-iodopyridine for B, pyridine and 1,3-dichloro-5-iodobenzene for D, and pyridine and 1,3-diiodobenzene for E). This procedure yields radicals that are low-energy species, as demonstrated, for example, by the lack of endothermic reactions.

Under typical FT-ICR conditions, the number of ions inside the cell is always much smaller than the number of neutral molecules, even at very low pressures (1×10^{-9} Torr). Under these conditions, the pseudo-first-order reaction rate law applies and can be used to obtain the second-order reaction rate constant from the measured pressure and the slope of a plot of ln(relative reactant ion abundance) versus reaction time. The pressure was measured by two ionization gauges located on each side of the dual cell. The ion gauge pressure readings were corrected for the sensitivity of the ion gauges toward each neutral reagent and for the pressure gradient between the ion gauge and the cell. The correction factors were obtained by measuring the reaction rate of an exothermic proton-transfer reaction from protonated acetone to the given neutral reagent. The reaction was assumed to occur at the collision rate. The accuracy of the measured rate constants is estimated to be about 50%, while the precision is better than 20%. The rate constants are reported as percent efficiencies of the reactions, which were obtained by dividing the second-order reaction rate constant (k_{exp}) by a calculated collision rate constant ($k_{theoretical}$). The collision rate constant was calculated using the parametrized trajectory theory of Su and Chesnavich.[19] A reaction with an efficiency of 1% means that only one out of 100 collisions of ions with reactant molecules leads to product formation.

Primary reaction products were identified based on their fixed relative abundances (branching ratios) at short reaction times. The primary product abundances are reported as percent branching ratios, which are given as the ratio of a primary product ion's abundance to the sum of all primary product ion abundances. When two or more reaction channels compete, the product branching ratios reflect the relative rates of the competing pathways. The estimated precision of the branching ratios obtained in these experiments is about 6% (absolute value).

The potential energy surfaces of hydrogen abstraction from αC and the NH_2 group of glycine by radicals A−E were explored at the UB3LYP/6-31G(d)//UHF/6-31G(d) level of theory by using the Gaussian 98 Revisions A.11 suite of programs.[20] In UHF optimization calculations, the zero Kelvin enthalpies of reactants, products, and transition states were calculated as the electronic energies corrected by the zero point vibrational energies. The zero point energies were scaled by multiplying by a scaling constant of 0.9135.[21] By performing frequency analysis, all optimized geometries were verified to be real local minima (no imaginary frequencies), and all transition states were

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Table 1. Efficiencies and Product Branching Ratios for the Reactions Studied[a]

amino acid		radical A EA = 6.2	radical B EA = 6.1	radical C EA = 5.8	radical D EA = 5.1	radical E EA = 4.9
glycine	NH_2 abstraction(%)	96	79	57	0	0
IE = 8.9	H abstraction (%)	4	21	43	100	100
PA = 211.9	efficiency (%)	50	21.5	8.5	0.3	0.3
2-d_2-glycine	NH_2 abstraction (%)	97.5	86.5	63.5	0	0
	H abstraction (%)	2	6.5	18.5	46	57
	D abstraction (%)	0.5	7	17.5	54	43
	efficiency (%)	53	25	9.5	0.13	0.14
alanine	NH_2 abstraction (%)	96	79	47	0	0
IE = 8.88	H abstraction (%)	4	21	53	100	100
PA = 215.5	efficiency (%)	53	45	26	1.0	1.2
valine	NH_2 abstraction (%)	87.5	proton	33	0	0
IE = 8.71	H abstraction (%)	12.5	transfer (100%)	67	100	100
PA = 217.6	efficiency (%)	60		64	8	4
proline	H abstraction (%)	100	proton	100	100	100
IE = 8.3	efficiency (%)	33	transfer (100%)	43	29	18
PA = 220.0						
cysteine	NH_2 abstraction (%)	16	proton	0	0	0
IE not known	H abstraction (%)	37	transfer (100%)	60	100	100
PA = 215.9	SH abstraction (%)	47		40	0	0
	efficiency (%)	66.5		71	31	23
methionine	NH_2 abstraction (%)	14	proton	0	0	0
IE = 8.3	H abstraction (%)	6	transfer (100%)	10	63.5	80
PA = 223.6	CH_3S abstraction (%)	80		86	32	17
	adduct-CH_3 (%)	1		3	4.5	3
	efficiency (%)	87		75	35	22

[a] Vertical electron affinities (EA in eV) of the radicals were calculated at the B3LYP/6-31+G(d) level of theory. The adiabatic first ionization energy (IE in eV) and proton affinity (PA in kcal/mol) of the amino acids were obtained from ref 22.

verified to be first-order saddle points (only one imaginary frequency corresponding to the dissociation of the transition state into the desired products). In DFT single point calculations, the zero Kelvin enthalpies were estimated as the DFT electronic energies corrected by the scaled UHF zero point energies.

Results and Discussion

General Radical Reactivity and Product Branching Ratios. The five positively charged phenyl radicals selected for this study are *N*-(2,3,5,6-tetrafluoro-4-dehydrophenyl)pyridinium (A), 3-dehydropyridinium (B), *N*-phenyl-3-dehydropyridinium (C), *N*-(3-chloro-5-dehydrophenyl)pyridinium (D), and *N*-(3-dehydrophenyl)pyridinium (E) (Figure 1). The product branching ratios and efficiencies of reactions with seven different amino acids (Figure 2) are shown in Table 1.

As has been reported earlier for reactions of charged phenyl radicals with simple organic substrates,[17] the radicals' electrophilicities (related to their calculated vertical electron affinities (EA) or the energy released upon attachment of an electron to the radical site) appear to play a major reactivity controlling role. In general, radicals with a greater EA react faster with the amino acids than radicals with a lower EA (Table 1). Hence, favorable polarization of the transition state appears to be a crucial controlling factor in these reactions. This finding parallels the discovery made by Donahue and Anderson [23,24] that the electronic structures of the transition states for a large set of neutral gas-phase radical substitution reactions are most strongly influenced by valence bond configurations that are ionic rather than neutral in nature and that estimates of barrier heights can be derived from purely ionic parameters of the system (EA of an electrophilic radical, IE of the substrate).

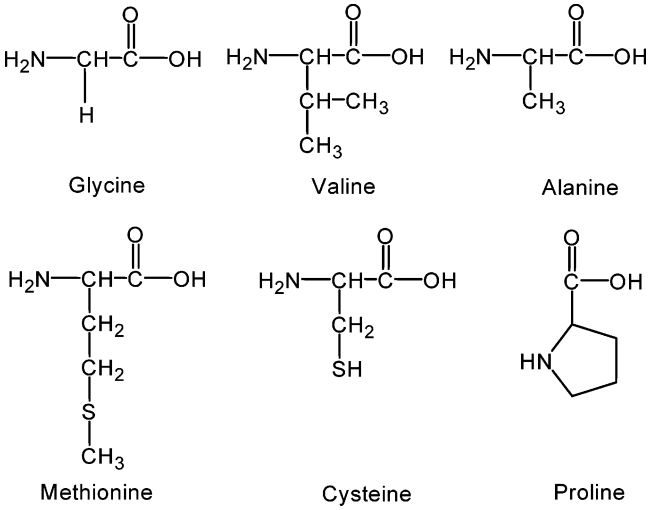

Figure 2. Structures of the amino acids used in this study.

The EA ordering of the radicals studied here (calculated at the B3LYP/6-31+G(d) level of theory) is as follows: radical A (6.2 eV) > radical B (6.1 eV) > radical C (5.8 eV) > radical D (5.1 eV) > radical E (4.9 eV). In most cases, this ordering also applies to the radicals' reactivity toward the amino acids studied. The amino acids can be separated into three categories based on the type of reactions they undergo (Table 1). The first

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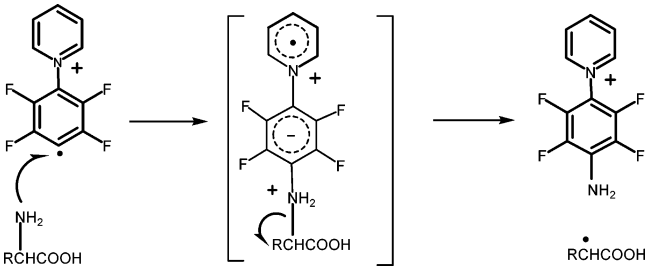

Figure 3. Possible mechanism for NH_2 abstraction from amino acids by radical A.

category includes the simplest amino acids, glycine, alanine, and valine. These amino acids react with all the radicals by either the expected hydrogen transfer or a novel NH_2 transfer or both (with the exception of radical B and valine: only proton transfer occurs). The second category consists of the only cyclic amino acid studied, proline (Figure 2), which reacts with radicals A−E (with the exception of radical B) only via hydrogen transfer. The third category contains cysteine and methionine for which additional, novel reaction pathways were observed. Cysteine transfers a SH group to several of the radicals. Methionine donates the CH_3S group to all of the radicals studied (except for B). The reactions of the different categories of the amino acids are discussed next.

Glycine, Alanine, and Valine. This group of amino acids displays two common pathways upon reaction with radicals A−E, namely, hydrogen abstraction and NH_2 abstraction. Radical B, the only Brönsted acid among the radicals studied, is an exception and reacts with valine, the most basic (PA = 217.6 kcal/mol, Table 1) among the three amino acids, by exclusive proton transfer. The less electrophilic radicals D and E react with these three amino acids only by hydrogen abstraction. However, the more electrophilic radicals A−C also react via NH_2-abstraction (with the exception of the reaction of B with valine). The greater the EA of the radical, the more favorable the reaction. Therefore, NH_2 abstraction may be best viewed as a nucleophilic addition−elimination reaction that likely proceeds through the formation of an addition intermediate (Figure 3). The structure of the addition intermediate of glycine and radical A shown in Figure 3 is supported by the calculated spin density distribution. It was found that the spin density is mainly centered in the pyridine ring at the 2- (0.283) and 4-positions (0.412) with respect to the nitrogen, which implies that the unpaired electron is delocalized over the pyridine ring. The formation of this intermediate is highly exothermic for all the radicals studied in the case of glycine. However, due to the high reaction exothermicity and the inability of the gas-phase system to disperse extra energy into a surrounding medium, the addition intermediate spontaneously fragments. A stable adduct was not observed for any of the radicals.

For a given radical, the larger the side chain of the amino acid, the higher the branching ratio of the hydrogen abstraction (Table 1). This phenomenon might imply that hydrogen abstraction occurs preferentially from the alkyl side chain of these amino acids.

According to the Anderson−Donahue avoided ionic curve crossing model,[23,24] the amino acids with lower ionization energies (IE) should react faster than those with higher IE values. This phenomenon was observed in reactions of all the radicals with this group of amino acids. For example, radical A reacts fastest with valine (IE = 8.71 eV), then alanine (IE = 8.88 eV), and slowest with glycine (IE = 8.9 eV).[22] The avoided curve crossing model also predicts a strong relationship between the EA of the radical and its reaction efficiency. This relationship was observed to apply to the reactions of radicals A−E with these three amino acids. For example, the most electrophilic radical (A) has the greatest efficiency of reaction with the three amino acids.

Proline. Proline is substantially more basic than the conjugated base of radical B. Therefore, proton transfer from radical B to this amino acid is so facile that it completely suppresses all possible radical reactions. Consequently, the following discussion focuses on the reactions of radicals A and C−E.

Proline cannot transfer an NH_2 group to a radical (Table 1) without ring-opening because this group is a part of a ring. Indeed, proline reacts with all the radicals only via hydrogen transfer (no fragmentation products indicating a ring-opening were observed). The ionic avoided curve crossing model predicts that proline (IE = 8.3 eV[22]) should undergo hydrogen transfer to any radical more rapidly than valine (IE = 8.71 eV[22]), which in turn should be more reactive than glycine and alanine. This indeed is the reactivity ordering of the amino acids toward radicals D and E, which only undergo hydrogen abstraction reactions.

Cysteine and Methionine. Cysteine and methionine are both basic (Table 1) and rapidly deprotonate the acidic radical B. The following discussion therefore only covers radicals A and C−E. The existence of an SH group in cysteine (Figure 2) has a strong influence on its reactivity. First, an abundant SH abstraction product was observed in reactions with the most electrophilic radicals A and C (Table 1). This unprecedented reaction pathway dominates the reactivity of radical A (which predominantly abstracts NH_2 from all the amino acids in the first category). Second, the presence of the SH group in cysteine, as opposed to SCH_3 in methionine, also dramatically increases the branching ratio of hydrogen abstraction by radical A. For other amino acids, this reaction channel is minor (except for proline where hydrogen abstraction is the only pathway). The increase of the product branching ratio of hydrogen abstraction from cysteine relative to that from methionine suggests that the majority of the abstracted hydrogen atoms comes from the SH group in cysteine. As mentioned in the Introduction, solution studies have demonstrated that SH in cysteine is the preferred site for hydrogen abstraction by the HO radical.[1]

SCH_3 abstraction is the predominant reaction pathway for the reactions of the most electrophilic radicals A and C with methionine, and it is one of the major pathways for the reactions of radicals D and E (that react exclusively via hydrogen abstraction with all the other examined amino acids). Different from the reactions with cysteine, hydrogen abstraction is a minor pathway for the reactions of radical A with methionine. This result provides further evidence in support of the sulfur hydrogen being the preferred site for hydrogen abstraction in cysteine.

Regioselectivity of Hydrogen Abstraction from Glycine. To determine which position in glycine is the preferred hydrogen abstraction site, glycine with the two αH atoms replaced with deuteriums was allowed to react with radicals A−E. The almost

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equal branching ratios for hydrogen and deuterium abstraction for radicals B−E (Table 1) demonstrate that the α carbon is not the only site in glycine from which a hydrogen atom is abstracted. A significant amount of hydrogen abstraction from the isotope-labeled glycine suggests that hydrogen atoms can also be abstracted from either NH_2 or OH in glycine. The αC−H bond in glycine (79.2 kcal/mol) is weaker than the N−H (102.6 kcal/mol) and O−H bonds (112.9 kcal/mol).[14] The likelihood for hydrogen abstraction from the OH group is small because of the large O−H bond dissociation energy. The branching ratios for hydrogen and deuterium abstraction products cannot be used directly to quantify the competition between hydrogen abstraction from αC and the NH_2 group because of a possible kinetic isotope effect. However, deuterium abstraction is usually slower than hydrogen abstraction. Therefore, the equal branching ratio of H and D abstraction implies that a larger amount of hydrogen atoms is abstracted from αC than from the NH_2 group in the nondeuterated glycine.

The potential energy surfaces for the hydrogen abstraction from both αC and the NH_2 group of glycine by radicals A−E were explored theoretically in this work. One of the main difficulties in the theoretical calculations of isolated amino acid molecules in the gas phase is the presence of numerous energetically close-lying conformers. To the best of our knowledge, comprehensive conformational studies (including both theoretical and spectroscopical methods) have been reported only for the simplest amino acids, including glycine, alanine, valine, serine, cysteine, proline, as well as the aromatic amino acid phenylalanine.[14,25−34] The conformational space of glycine in the gas phase has been fully explored.[14,25] At 0 K, only the most stable conformer of glycine exists.[25] At room temperature (normal FT-ICR conditions), the most stable conformer accounts for about 70% of the total equilibrium composition of glycine.[25] In the present study, all molecular orbital calculations were carried out on the most stable conformer of the neutral glycine molecule and the relevant glycine radicals (Figure 4).

The geometries of the transition states for hydrogen abstraction from αC and the NH_2 group of glycine by radicals A and B calculated at the UHF/6-31G(d) level of theory are illustrated in Figure 4. The geometries of the corresponding transition states for the other radicals studied are similar to those of radical B and hence omitted. The computational results are given in Table 2. For gas-phase ion molecule reactions, the activation energies are normally negative relative to the separated reactants due to stabilization of the ion by the neutral molecule. For a given radical, the transition state energy (relative to the separated reactants) of hydrogen abstraction from αC is 2−3 kcal/mol lower than that of hydrogen abstraction from the NH_2 group. Therefore, hydrogen abstraction from αC is kinetically favored over that from the NH_2 group. The $^{\bullet}NHCH_2COOH$ radical is calculated to be 18.5 kcal/mol less stable than the $NH_2CH^{\bullet}$-COOH radical at the B3LYP/6-31G(d)//UHF/6-31G(d) level of theory, in agreement with the reported 23.4 kcal/mol bond dissociation energy (BDE) difference of NH−H and αC−H, and the theoretically predicted BDE difference of 19.9 kcal/mol obtained at the G2(MP2) level of theory.[14] Therefore, for a given radical, hydrogen abstraction from αC is also thermodynamically favored over hydrogen abstraction from the NH_2 group (Table 2).

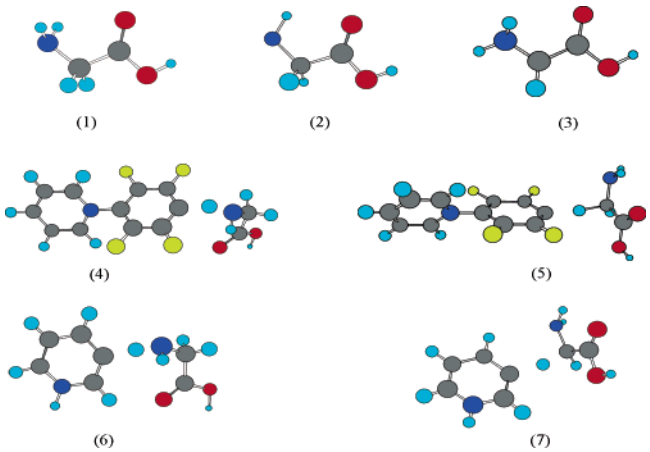

Figure 4. Geometries of the most stable conformers of glycine (**1**), $^{\bullet}NHCH_2COOH$ radical (**2**), $NH_2CH^{\bullet}COOH$ radical (**3**), and the geometries of the transition states of hydrogen abstraction from NH_2 (**4** and **6**) and αC (**5** and **7**) of the most stable conformer of glycine by radicals A and B, respectively. All geometries were optimized at the UHF/6-31G(d) level of theory.

Table 2. Transition State Energies (Relative to the Separated Reactants and Therefore Negative) and Reaction Enthalpy Changes for Hydrogen Abstraction from αC and NH_2 of Glycine by Radicals A−E, as Calculated at the UB3LYP/6-31G(d)//UHF/6-31G(d) Level of Theory[a]

radical A	radical B	radical C	radical D	radical E
transition state energies of hydrogen abstraction from αC				
−15.2	−14.8	−13.8	−8.5	−7.6
enthalpy changes of hydrogen abstraction from αC				
−39.3	−39.2	−36.9	−33.5	−33.3
transition state energies of hydrogen abstraction from the NH_2 group				
−12.2	−12.9	−11.6	−6.2	−5.6
enthalpy changes of hydrogen abstraction from the NH_2 group				
−20.8	−20.6	−18.3	−15.0	−14.8

[a] Values shown are given in kcal/mol with respect to the separated reactants.

In general, our molecular orbital calculations indicate that hydrogen abstraction occurs preferentially from αC. Hydrogen abstraction from the NH_2 group is likely to be a minor reaction channel, although a hydrogen abstraction product was observed in the reactions of 2-d_2-glycine with radicals **A**−**E**. For hydrogen abstraction from both αC and NH_2, the transition state energy ordering for different radicals is A ≈ B < C ≪ D < E, in agreement with the prediction based on the ionic avoided curve crossing model.[23,24]

Conclusions

In this work, the gas-phase reactions of several amino acids with phenyl radicals A−E were investigated. In addition to

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hydrogen atom abstraction, an unprecedented abstraction of various groups was also observed. These include NH_2 group abstraction from most amino acids, SH group abstraction from cysteine, and SCH_3 group abstraction from methionine. For the most electrophilic radicals (A−C), group abstractions correspond to the major reaction pathways. These reactions likely do not proceed via a radical mechanism but rather through a nucleophilic addition−elimination pathway.

The ionic avoided curve crossing model[23,24] can be used to predict the order of the radicals' reactivity toward the amino acids. The electron affinity of the radical and the ionization energy of the amino acid are the major factors in controlling the radical's relative reaction rates with each amino acid.

For simple amino acids, the product branching ratio of hydrogen abstraction increases relative to NH_2 abstraction when the size of the side chain of the amino acid increases. This may imply that a certain amount of hydrogen atoms is abstracted from the alkyl side chain of the amino acid. A large increase in the hydrogen abstraction efficiency in the reactions of the radicals with cysteine suggests that a large portion of the abstracted hydrogen atoms comes from the SH group. Results obtained by examining the reaction of isotope-labeled glycine with radicals A−E and by theoretical calculations suggest that a large amount of hydrogen atoms is abstracted from αC and a small amount from somewhere else (likely the NH_2 group).

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